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<b>(21) International Application Number:</b> PCT/GB98/01753 <b>(22) International Filing Date:</b> 16 June 1998 (16.06.98) <b>(30) Priority Data:</b> 97304295.5 19 June 1997 (19.06.97) EP <b>(34) Countries for which the regional or international application was filed:</b> AT et al. <b>(71) Applicant (for all designated States except US):</b> UNICHEMA CHEMIE B.V. [NL/NL]; Buurtje 1, NL-2802 BE Gouda (NL). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> LINDNER, Nigel [GB/GB]; Meadow Walk, High Street, Little Addington, Northamptonshire NN14 4BD (GB). BOSLEY, John [GB/GB]; 43 Kettering Road, Islip, Kettering, Northamptonshire NN14 3JT (GB). MACRAE, Alasdair [GB/GB]; Ivy Cottage, Newton Blossomville, Bedford MK43 8AN (GB). SVENSSON, Ingemar [SE/SE]; Jurgvagen 75, S-226 57 Lund (SE). <b>(74) Agent:</b> KEITH W. NASH & CO.; 90-92 Regent Street, Cambridge CB2 1DP (GB).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> POLYMER COMPRISING UNSATURATED ESTER UNITS AND PHARMACEUTICAL AND COSMETIC COMPOSITIONS THEREOF <b>(57) Abstract</b> <p>Disclosed is a polymeric molecule comprising a plurality of repeat units having the structure <math>-(O_2C-R-CO_2)-</math>, wherein R is a substituted or unsubstituted, unsaturated hydrocarbon radical having 14 or more carbon atoms. Also disclosed are pharmaceutical and cosmetic compositions comprising a polymer having a plurality of repeat units having the structure <math>-(O_2C-R-CO_2)-</math>, wherein R is a substituted or unsubstituted, unsaturated hydrocarbon radical having 10 or more carbon atoms, and various uses of said compositions.</p>		

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**Title:**        Improvements in or Relating to Polymeric Compositions

**Field of the Invention**

This invention relates to certain polymeric molecules, compositions comprising polymeric molecules, a method of making said compositions, and to methods of treating a human subject.

**Background of the Invention**

Saturated dicarboxylic ("dioic") acids and their derivatives are compounds which are noted for their antimicrobial activity and for their beneficial effects on human skin. In particular the C<sub>9</sub> saturated dioic acid ("azelaic" acid), having the formula COOH-(CH<sub>2</sub>)<sub>7</sub>-COOH, is frequently cited as being effective in the treatment of acne and other skin conditions, and in the lightening of skin. Dioic acid derivatives which are suggested to be useful include salts, esters, amides, and mercapto-compounds (e.g. US 4,818,768, US 4,292,326, EP 0,297,436, EP 0,305,407, JP 58-170713).

The prior art teaches that conditions susceptible to treatment with dioic acids include: acne (US 4,386, 104); wrinkles (EP 0,336,880); malignant melanoma (US 4,818,768); dermatoses (EP 0,229,654); hyperpigmentary dermatosis and eczema (US 4,292,326); rosacea (EP 0,890,308); lentigo (JP 91024412) and seborrhoea (DE 3133425) and impetigo.

WO 94/07837 teaches that unsaturated dioic acids and derivatives thereof are generally more effective than their saturated counterparts, having greater anti-microbial and skin-lightening activity. WO 94/07837 also discloses a microbial method of making unsaturated dioic acids. There is no disclosure of polymers comprising unsaturated dioic acids.

WO 94/12652 discloses a method of producing a polyester, comprising as a repeating unit

"at least one aliphatic dicarboxylic acid". The document states (page 5) that: "suitable aliphatic dicarboxylic acids include those of formula  $\text{HO}_2\text{C}-\text{R}^5-\text{CO}_2\text{H}$  wherein  $\text{R}^5$  is a bond or is a divalent radical defined as for  $\text{R}^1$ " ( $\text{R}^1$  having been previously defined in the document as a "substituted or unsubstituted  $\text{C}_1$  to  $\text{C}_{12}$  alkyl group optionally having one or more carbon-carbon double bonds..."). The polyesters produced by the process described in WO 94/12652 are said to "find uses as shaped articles and foams, particularly for motor vehicles". There is no suggestion of the use of polymers comprising dioic acids in any other technical fields.

### Summary of the Invention

In a first aspect the invention provides a polymeric molecule comprising a plurality of repeat units having the structure  $-(\text{O}_2\text{C}-\text{R}-\text{CO}_2)-$ , wherein R is a substituted or unsubstituted, unsaturated hydrocarbon radical having 13 or more carbon atoms. It is preferred that the polymer is a lipase-degradable polyester, which upon exposure to a lipase releases a compound having a beneficial effect on a human subject. The released compound will therefore be a substituted or unsubstituted, unsaturated dioic acid.

In a second aspect, the invention provides a composition suitable for topical application to the human body, the composition comprising: a polymeric molecule having a plurality of repeat units having the structure  $-(\text{O}_2\text{C}-\text{R}-\text{CO}_2)-$ , wherein R is a substituted or unsubstituted, unsaturated hydrocarbon radical having 10 or more carbon atoms; and an acceptable carrier substance. Preferably the composition comprises a polymeric molecule in accordance with the first aspect defined above. Such compositions have unexpected properties and are suitable for a wide range of purposes, discussed further below.

Preferably, in the repeat units of the polymeric molecule R is aliphatic and advantageously comprises 16 to 20 carbon atoms (such that the repeat units themselves comprise 18-22 carbon atoms), and preferably three, or more preferably two or one, carbon-carbon double bonds, which repeat units (derived from dioic acids) appear to have optimum anti-microbial activity when released from the polymer by lipase-mediated degradation. In preferred embodiments, R will comprise a C16:1, C16:2, C16:3, or a C20:1 moiety (the repeat units therefore being C18:1, C18:2, C18:3, or C22:1 units which may conveniently

be respectively obtained from oleic, linoleic, linolenic or erucic acids).

Advantageously, the composition of the invention will comprise the preferred embodiments, defined above, of the polymeric molecule of the first aspect of the invention.

Those skilled in the art will appreciate that a number of compounds could be used as reagents with dioic acids to form polymeric molecules as defined above. For instance, the polymeric molecule could be a homopolymer, consisting of repeat units of identical dioic acid monomers. Alternatively, a co-polymer could be formed from mixtures of two or more different dioic acids (such as combinations of unsaturated dioic acids with saturated dioic acids, or combinations of unsaturated dioic acids of different chain lengths).

Conveniently, the polymeric molecule comprises a co-polymer of a dioic acid esterified with one or more di- or polyols. Examples are glycerol and butanediol, which have certain useful properties beneficial to the skin, and/or are particularly convenient in the preparation of useful compositions. Other polyols useful in the preparation of polymeric molecules are  $C_6$ - $C_{10}$  polyols, which are those most readily miscible with the preferred dioic acid monomers.

The preferred average molecular weight of the polymeric molecule will also depend, in part, on the intended use of the composition comprising the polymeric molecule. The lower and upper limits are around 1kDa and 50 kDa respectively. A polymer of around 1kDa molecular weight will have about 3 or 4 repeat units. The generally preferred range is 3.5-10kDa. Polymers of this size are generally liquids and thus more readily processed.

The compositions of the invention have a considerable number of possible uses, all of which are considered as being within a further aspect of the invention. The nature and structure of the polymeric molecules can be tailored to the intended purpose of the composition. Indeed, in some circumstances it will be advantageous if the composition comprises two or more distinct polymeric molecules of the type defined above with complementary characteristics.

The compositions of the invention are formulated for topical application to the human body, advantageously being suitable for application to the skin, scalp or hair. Generally it is envisaged that, as with the compositions disclosed in WO 94/07837, the compositions of the present invention are useful as cosmetic compositions, improving skin quality and complexion, and as pharmaceutical compositions having anti-microbial effects (which can also enhance skin condition). Specifically, the compositions of the invention may be of use in the treatment of: acne; wrinkles; malignant melanoma; dermatoses: hyperpigmentary dermatosis and eczema; rosacea; lentigo, seborrhoea and impetigo; and in the treatment of body malodour. Additionally, the compositions may be used for the lightening of skin. In particular, the compositions are useful as anti-perspirants and/or deodorants.

The compositions may *inter alia*, take the form of gels, creams, lotions, oils or waxes (hard or soft).

Advantageously the composition will be buffered to the range pH 4-7, preferably within the range pH 5.5-6.5, at which pH the dioic acid (or derivative) monomeric units released by the action of a lipase appear to exert optimum anti-microbial effect.

Once applied to the skin, the polymeric molecules present in the composition are subject to degradation mediated by lipases present on the surface of the human body. Generally such lipases are secreted by microorganisms which colonise the skin. Such lipase-mediated degradation leads to the release of dioic acid monomer units (or substituted derivatives thereof) from the polymeric molecule, which monomer units have desirable cosmetic and antimicrobial properties. Thus, there is controlled release of active agent from the polymeric molecule, which occurs most rapidly in response to high concentrations of lipases, which will be present at sites of maximum microbial colonisation (e.g. spots, locally inflamed areas, skin pores).

Most lipases have greater freedom to act at the ends of polymeric molecules rather than in the middle of the polymer chain, where steric considerations can inhibit the action of the enzyme. Accordingly, the concentration of "free ends" available for lipase-mediated

degradation is relatively greater for a composition comprising short polymeric molecules than for compositions comprising long polymeric molecules. Also, the lower molecular weight polymers are thought to be less crystalline in structure, and so more accessible to lipases. It will therefore be advantageous for some uses (e.g. as topically-applied cosmetic agents) if compositions in accordance with the invention comprise polymeric molecules with a range of molecular weights, such that smaller polymers will be present for relatively rapid degradation, giving relatively quick release of active agent, whilst larger polymers which are degraded more slowly are included to give a longer lasting supply of active agent. The choice of polymer size can therefore be tailored to suit the intended purpose, and/or frequency of application, of the composition.

The present inventors expect that compositions in accordance with the present invention have surprising properties which make them suitable for many other uses. Suitable compositions can form occlusive, moisture-retaining films when applied to the skin. Thus the compositions are particularly useful in barrier creams, and in "after sun" creams, gels and lotions and as anti-perspirants/deodorants. In addition the compositions may be used to form clear or translucent dermal patches, which can comprise other substances which are desired to be delivered transdermally (e.g. nicotine).

Where it is desired to maximise the occlusive properties of the composition, it will generally be preferred to use a longer chain dioic acid repeating unit and/or a longer chain alcohol, to increase the hydrophobicity of the resulting polymeric molecule.

The occlusive, anti-microbial characteristics of compositions in accordance with the invention renders them ideal for use in dressings to promote wound healing (especially healing of burns, where both moisture-retention and anti-microbial properties are desired). Thus, in another aspect, the invention provides a medical dressing (such as a bandage, plaster, mesh, wound dressing etc.) for application to the skin of a human subject, the dressing comprising a dressing substrate impregnated or coated with a polymeric molecule in accordance with the invention.

Suitable formulations for the compositions of the invention will be apparent to those

skilled in the art and include those, for example, taught by US 4,818,768.

In particular it will be preferred that compositions for direct application to the skin will comprise a relatively volatile organic solvent (such as lower alcohols,  $C_2$ - $C_4$ ). Mixing an organic solvent with the polymeric molecule causes a substantial decrease in the viscosity of the composition, thereby facilitating application of the composition as a liquid or a spray: after application, a relatively volatile organic solvent will evaporate, leaving a film of the polymeric molecule (which may subsequently be hydrolysed by lipases).

The polymeric molecules for inclusion in the composition of the invention may be synthesised by standard chemical methods, or may be produced via biocatalytic methods, as described in the examples below and in WO 94/12652. Suitable lipases are also disclosed therein. Lipases obtainable from *Candida antarctica* are particularly suitable. Biocatalytic methods are generally to be preferred when forming polymers comprising polyols, as the substrate specificity of lipases ensures a greater degree of uniformity of product (with reaction mainly at the primary -OH groups) than that obtainable by conventional chemical methods. In particular, the use of an enzyme avoids excessive cross-linking (with reaction occurring mainly at the primary -OH groups), ensuring an essentially linear, bio-degradable polymer. In contrast, conventional chemical methods can result in excessive cross-linking, leading to highly branched products which tend to precipitate out of the reaction mixture in an uncontrolled manner. In addition, when the polymers are synthesised by use of a lipase, it increases the likelihood that the polymeric product will have the desirable property, in the final composition, of being lipase-degradable.

The invention also includes within its scope the use of the composition defined above as a cosmetic and/or therapeutic composition; and a method of making the composition defined above.

Accordingly the invention provides a method of treating the skin of a human subject, comprising applying to the skin a composition in accordance with the second aspect of the invention, the composition comprising a polymer having a plurality of repeat units having



the structure  $-(O_2C-R-CO_2)-$ , wherein R is a substituted or unsubstituted, unsaturated hydrocarbon radical having 10 or more carbon atoms, said polymer being lipase-degradable to release an active substance having a beneficial anti-microbial and/or cosmetic effect. Conditions treatable by the method are as explained above. Preferably the method of treating skin will comprise the use of a composition comprising a polymer in accordance with the first aspect of the invention.

The invention also provides a method of making a cosmetic or pharmaceutical composition suitable for topical application to a human subject, the method comprising mixing an effective amount of a polymer having a plurality of repeat units having the structure  $-(O_2C-R-CO_2)-$ , wherein R is a substituted or unsubstituted, unsaturated hydrocarbon radical having 10 or more carbon atoms, with an acceptable carrier substance (e.g. aqueous solutions, organic solvents, emollients, emulsifiers, or mixtures of any of the foregoing). Desirably the method will comprise the use of a polymer in accordance with the first aspect of the invention.

In particular, the invention provides a method of making a medical dressing (such as a bandage, wound dressing, plaster, mesh or the like) for application to the skin of a human subject, the method comprising coating or impregnating a suitable dressing substrate with a polymer having a plurality of repeat units having the structure  $-(O_2C-R-CO_2)-$ , wherein R is a substituted or unsubstituted, unsaturated hydrocarbon radical having 10 or more carbon atoms, to form a dressing. Desirably the medical dressing will be coated or impregnated with a polymer in accordance with the first aspect of the invention. Once made, the dressing will conveniently be packaged and sterilised (e.g. by irradiation).

In a further aspect the invention provides for use of a lipase-degradable polymer in the manufacture of a pharmaceutical composition for the treatment of a human skin condition (e.g. acne, pimples and the like) caused or exacerbated by, or associated with, microbial growth, the polymer having a plurality of repeat units having the structure  $-(O_2C-R-CO_2)-$ , wherein R is a substituted or unsubstituted, unsaturated hydrocarbon radical having 10 or more carbon atoms. Advantageously the polymer will be in accordance with the first aspect of the invention.

The invention will now be further described by way of illustrative example and with reference to the accompanying drawings, in which:

Figure 1 is a graph of total viable count (TVC) against time (hours) for *Corynebacteria* in the presence of: (i) no added substance (negative control, filled squares); (ii) added C18:1 dioic acid/butanediol polyester (MW 4,800, filled diamond symbols); and (iii) added free C18:1 dioic acid (filled star symbols);

Figure 2 is a graph of total viable count (TVC) against time (hours) for *Corynebacteria* in the presence of a composition in accordance with the invention with (filled diamond symbol) or without (filled square symbol) exogenously added lipase; and

Figure 3 is a graph of total viable count (TVC) against time for *Corynebacteria* in the presence of one of four different compositions in accordance with the invention, having respective molecular weights of 4,800 (filled squares); 16,600 (filled diamonds); 34,700 (filled stars); or  $\approx$  4,600 (open squares).

**Example 1** - Synthesis of a co-polymer comprising, as monomers, C<sub>18:1</sub> dioic acid and butan-1, 4-diol.

**Materials:**

9-octadecen-1, 18-dioic acid (C18:1 DCA), ex Unichema, 91.66 g.

1,4-butanediol, ex Fluka, 26.35 g.

Immobilised *Candida antarctica* B lipase, SP 435 (Novo Nordisk, Denmark), 400 mg.

**Reaction:**

The substrates were heated to 60°C in a 150 ml glass reactor vessel and stirred at 200 r.p.m. with an overhead stirrer fitted with a Teflon paddle. The dicarboxylic acid melted and dissolved in the butanediol, the reaction was then started by adding the immobilised lipase preparation. After 24 hours reaction the pressure in the head space of the reactor was lowered to 50 mbar, by connecting a vacuum-pump. The water formed during the reaction was then removed under vacuum to force the chemical equilibrium towards ester

formation and to increase the molecular weight of the polyester product. After further 24 hours under vacuum the reaction was stopped and the lipase catalyst was removed by vacuum-filtration on a filter paper at 60°C.

**Analyses:**

The reaction was followed by taking samples and titrating the free carboxylic acid groups with NaOH-solution using phenolphthalein as indicator. A sample of 0.2-0.4 g was dissolved in 20 ml diethylether/ethanol (1:1) and titrated with 100 mM NaOH in water/ethanol (1:1) until a colour change was noted.

The increase in polymer molecular weight was followed by Gel Permeation Chromatography (GPC) on one TSK 1000 and one TSK 3000 column from Supelco used in series at 30°C. Solvent used was tetrahydrofuran (THF) at a flow-rate of 1 ml/min. A differential refractive index detector (Gilson) was used as detector. Calibration was done with commercial polystyrene standards except where otherwise indicated. The mass- and number-average molecular weights ( $M_w$  and  $M_n$ ) were calculated by use of a computer program (TURBOGEL™) from P E Nelson.

**Results:**

The polyester produced under the conditions described above was an amber-coloured, viscous liquid at room temperature. The  $M_w$  was 4800, polydispersity ( $M_w/M_n$ ) 2.2 and the acid value (mg KOH/g sample) 27.8.

By repeating the reaction with more catalyst and/or longer reaction times higher molecular weights of polyester were achieved. The products were all liquids and showed increasing viscosity with increasing molecular weight. Their properties are summarised in Table 1, below.

**Table 1** Polyesters formed from C18:1 DCA and 1,4-butanediol

Reaction	Mw	Polydispersity	Acid value
1	4800	2.2	27.8
2	16600	2.3	22.7
3	34700	3.8	8.0
4*	47400	2.0	9.0

\* This preparation contained some THF-insoluble material.

### Example 2

The butanediol monomer was substituted with glycerol and using the 1,3-specific lipase from *Rhizomucor miehei* (Lipozyme ex Novo Nordisk, Denmark). A non-crosslinked polyester containing hydroxyl side groups was formed.

#### **Materials:**

9-octadecen 1,18-dioic acid, ex Unichema, 50.0 g.

glycerol, 99% ex Aldrich, 14.7 g.

distilled water, 645  $\mu$ l.

Immobilised *Rhizomucor miehei* lipase (Lipozyme IM60), 2.0 g.

#### **Reaction:**

The reaction was performed as described in Example 1, except that the initial vacuum applied was 100 mbar. This vacuum was gradually lowered during the course of the reaction to 50 mbar and finally to 11 mbar. The reaction was stopped after 4 days and the catalyst removed by vacuum filtration at 80°C. The polyester produced was a translucent viscous liquid. Mw(PEG-Standard) 4600, Polydispersity 3.3 and the acid value 33.1.

### Example 3

#### **Materials:**

Mixed medium chain monounsaturated dicarboxylic acid, 25.00 g.

[Composition. main peaks in gas chromatography: C6:0 DCA-1.2%, C8:0 DCA-7.2%, C10:1 DCA-16.1%, C10:0 DCA-5.0%, C12:2 DCA-0.5%, C12:1 DCA-38.0%, C12:0 DCA-4.6%, C14:2-2.9%, C14:1 DCA-18.7%, produced by the fermentative method disclosed in WO 94/07837.]

1,4-butanediol, ex Fluka, 10.01 g.

Immobilised *Candida antarctica* B lipase, SP 435, ex Novo Nordisk, 1.00 g.

#### **Reaction:**

The reaction was performed as in Example 1, except that the reaction temperature used was 70°C and the vacuum (100 mbar) was not applied until the end of the reaction at six days to remove condensates in the reactor vessel. The product was isolated by vacuum filtration at 70°C to give a yellow oil having an acid value of 71.6, Mw of 4800 and polydispersity 3.5.

### Example 4

#### **Materials:**

9-octadecen 1,18-dioic acid, ex Unichema, 25.00 g.

1,12-dodecanediol, ex Fluka, 14.98 g.

Immobilised *Candida antarctica* B lipase, SP 435 (Novo Nordisk, Denmark), 1.00 g.

#### **Reaction:**

The reaction was performed as in Example 1, except that the reaction temperature used was 70°C, in order initially to melt the reaction mixture, and the vacuum of 100 mbar was applied after 170 minutes and maintained until the end of the reaction at 22 hours. To remove catalyst from the products of the reaction a solvent, methylethylketone, was used to lower the viscosity and this slurry was vacuum-filtered at 70°C. The solvent was removed at 90°C under vacuum. The product was a wax and had an acid value of 23.5,

Mw of 18500 and polydispersity 4.0.

### **Example 5**

#### **Materials:**

9-octadecen 1,18-dioic acid, ex Unichema, 15.00 g.

1,12 dodecanedioic acid (C12:0 DCA), ex Aldrich, 9.905 g.

1,4-butanediol, ex Fluka, 7.752 g.

Immobilised *Candida antarctica* B lipase, SP 435 (Novo Nordisk, Denmark), 1.00 g.

#### **Reaction:**

The reaction was performed as in Example 1, except that the reaction temperature used was 70°C. Initially the reaction was started with only C18:1 DCA and the butanediol. After 1 hour of reaction, when the formation of some low molecular weight polymers and oligomers had occurred, the C12:0 DCA was added. A vacuum of 100 mbar was slowly applied to the head space at 3 hours and maintained until the end of the reaction at 22.5 hours. After six hours the reaction mixture became homogeneous. The lipase catalyst was removed by vacuum-filtration at 70°C. The product was a soft wax at room temperature, and had an acid value of 23.5, Mw of 10400 and polydispersity 3.5.

### **Example 6 - Measurement of antimicrobial properties**

#### **Material:**

*Coryneform (G42) bacterium*: is an isolate from human skin at Colworth Laboratories, Bedford, UK. It is a Gram +ve and lipase-producing organism. Corynebacteria are commonly found on human skin and are associated, amongst other things, with the causation of body malodour.

*Polyester emulsion*: the polyester prepared in Example 1, 1 g/l, was aseptically emulsified with 5 g/l Triton X100 in 10 mM, pH 6, Na-phosphate buffer.

*Growth medium*: 20 g/l CASO Bouillon, Merck; 10 g/l Yeast extract, Beta lab, UK; 2.5 g/l Tween 80, Sigma; Millipore water.

*Agar plates*: 30 g/l CASO Bouillon, Merck; 10 g/l Yeast extract, Beta lab, UK; 10 g/l

Tween 80, Sigma: 20 g/l Agar, Oxoid; Millipore water.

### Methods:

*To test the effect of the made polyesters on Corynebacteria cells:* A suspension of freshly grown viable *Corynebacteria* cells were incubated in 30 ml polyester emulsion in shaking flasks at 35°C. Samples of 100 µl were taken at different times and Total Viable Count (TVC) of the cells was made. A cell suspension without polyester, and one with added free C18:1 dicarboxylic acid (at 0.5 gms/l), were treated in exactly the same way to act as negative and positive controls respectively.

*Total Viable Count:* After diluting the sample in Ringer's solution and making a dilution series, 100 µl from each dilution was applied and spread on to agar plates. These plates were then incubated at 35°C for two days or until the growing colonies were big enough to count.

### Results

Table 2

Total Viable Count (cells / ml)

Contact time (hours)	Cell control	Cells + polyester (MW 4,800)	Cells + free C18:1 DCA (0.5gms/l)
0	$2.5 \times 10^9$	$2.5 \times 10^9$	$2.5 \times 10^9$
0.25	-	-	$4.7 \times 10^8$
1	-	-	$1.0 \times 10^7$
2	-	$1.0 \times 10^8$	-
5	-	6,000	40
8	-	110	-
24	$3.3 \times 10^8$	10	10

These results are also shown graphically in Figure 1. The data show that over a 24 hr period the number of viable cells incubated in the absence of polyester or free dioic acid was essentially stable, with less than a log decrease in cell number. In contrast, cell

viability is essentially lost in the presence of either polyester or free dioic acid. In the presence of free acid, a  $>2$  log reduction occurred within the first hour. In the presence of polyester (MW 4,800) there is a slower loss of viability ( $>1$  log in 2 hours), presumably because the polyester must first be hydrolysed to give the active anti-microbial substance. However, the final viable count was virtually zero, as with the free acid-treated cells.

#### Example 7 - Antimicrobial properties in presence or absence of added lipase

This study was performed essentially as in example 6 above.

#### **Result:**

*Corynebacteria* were incubated with a polyester emulsion made with a C18:1 DCA-butanediol polyester with  $M_w=4800$ , as described in Example 1. In one test extra lipase was added (10  $\mu$ l of Lipozyme 10 000L, Novo) together with the bacterial cells. The results are shown in Table 3 below, and graphically in Figure 2.

Contact time (hrs)	TVC (viable cells/ml)	
	cells	cells + lipase
0	$2.7 \times 10^9$	$3.4 \times 10^9$
1	$3.0 \times 10^9$	$4.1 \times 10^7$
2	$1.4 \times 10^9$	$< 1 \times 10^4$
4	$1.0 \times 10^7$	$< 1 \times 10^4$
7	$< 1 \times 10^3$	$< 1 \times 10^3$
25.5	$< 1 \times 10^2$	$< 1 \times 10^2$

**Table 3** Effect of polyester emulsion on the TVC of *Corynebacteria* cells both without and with extra lipase added.



The addition of free lipase to the test system substantially increases the rate of loss of viability, due to the increased rate of hydrolysis of the polyester. This indicates that active compounds will be generated most rapidly at sites with high concentrations of lipase which, in turn, will be at sites of maximum microbial colonisation.

#### **Example 8 - Effect of polyesters of different molecular weight**

This example relates to comparison of the effect of different molecular weight polyesters on *Corynebacteria* cells. This study was performed essentially as in Example 7. The polyesters used from Example 1 had Mw's of 4800, 16,600 and 34700. Additionally, the polyester from Example 2 with C18:1-glycerol was investigated in this example. The results are shown below in Table 4, and represented graphically in Figure 3.

Contact time (hrs)	butanediol polymer Mw = 4800	butanediol polymer Mw = 16600	butanediol polymer Mw = 34700	glycerol polymer Mw - "4600"
0	$3.2 \times 10^9$	$3.2 \times 10^9$	$3.2 \times 10^9$	$3.2 \times 10^9$
1	$2.3 \times 10^9$	$3.0 \times 10^9$	$2.6 \times 10^9$	$3.1 \times 10^9$
2	$1.6 \times 10^9$	$3.2 \times 10^9$	$2.9 \times 10^9$	$2.2 \times 10^9$
4	$6.8 \times 10^7$	$0.96 \times 10^9$	$2.3 \times 10^9$	$7.5 \times 10^8$
6	$1.4 \times 10^5$	$1.5 \times 10^8$	$1.2 \times 10^9$	$7.5 \times 10^7$
23	< 10	< 10	< 10	< 10

**Table 4** Effect of different polyester emulsions on the TVC of *Corynebacteria* cells.

These data show that as the molecular weight of the C18:1 dioic acid/butanediol polyester is increased, the initial rate of antimicrobial activity is decreased because there is a lower concentration of "free ends" of polymer molecules available for lipase-mediated degradation of the free acid. The rate of loss of cell viability is lower with the 4,600 MW

glycerol-based polyester than with the equivalent molecular weight butanediol-based polyester. This is presumably due to slower hydrolysis, which in turn is probably a result of steric hindrance problems caused by the presence of limited esterification at the secondary -OH group of the glycerol molecule. However, the polymer is still degradable.

Figure 3 shows the results for the initial 6 hours of the experiment. The final time point has been omitted from the graph to emphasise the difference in initial rates of hydrolysis (after 23 hours' incubation the cell viability is  $< 10$  cells/ml for all the polymers used). Overall, the experiment shows that the nature of the polymeric molecule can be altered to affect the rate of release of the active substance.

#### Example 9

This example investigates the effect of a polyester emulsion with mixed medium mono-unsaturated-DCA (with average molecular weight of about 4,800) from Example 3 on *Corynebacteria* cells. This study was performed as described in Example 5. The results are shown below in Table 5.

Contact time (hrs)	Viable cells/ml
0	$4.0 \times 10^9$
1	$2.3 \times 10^9$
2	$1.2 \times 10^9$
5	$4.5 \times 10^7$
24	$4.8 \times 10^3$

**Table 5** Effect of polyester emulsion on TVC of *Corynebacteria* cells.

**CLAIMS**

1. A polymeric molecule comprising a plurality of repeat units having the structure  $-(O_2C-R-CO_2)-$ , wherein R is a substituted or unsubstituted, unsaturated hydrocarbon radical having 14 or more carbon atoms.
2. A molecule according to claim 1, wherein R is aliphatic.
3. A molecule according to claim 1 or 2, wherein R comprises 16 to 20 carbon atoms.
4. A molecule according to any one of the preceding claims, wherein R is mono-, di- or tri-unsaturated.
5. A molecule according to any one of the preceding claims, wherein R is a C16:1, C16:2, C16:3 or C20:1 moiety.
6. A polyester according to any one of the preceding claims.
7. A polyester according to claim 6, wherein the polymer is lipase-degradable and, upon exposure to a lipase, releases a compound having an anti-microbial effect.
8. A polymer according to any one of the preceding claims formed at least in part by the polyesterification of an unsaturated dioic acid with glycerol or butandiol.
9. A polymer according to any one of the preceding claims having an average molecular weight in the range 3.5-10kDa.
10. A composition suitable for topical application to the human body, the composition comprising: a polymeric molecule having a plurality of repeat units having the structure  $-(O_2C-R-CO_2)-$ , wherein R is a substituted or unsubstituted, unsaturated hydrocarbon radical having 10 or more carbon atoms; and an acceptable carrier substance.

11. A composition according to claim 10, comprising a polymeric molecule in accordance with any one of claims 1 - 9.
12. A composition according to claim 10 or 11, comprising a plurality of different polymeric molecules, each being in accordance with any one of claims 1-9.
13. A composition according to any one of claims 10 - 12, formulated for use as a pharmaceutical or cosmetic.
14. A composition according to any one of claims 10 - 13, being buffered to a pH in the range 4-7, preferably pH5.5-6.5.
15. A composition according to any one of claims 10 - 14, comprising one or more of the following: a volatile organic solvent, an emollient, or an emulsifier.
16. A composition according to any one of claims 10 - 15, for use in the formation of a moisture-retentive, occlusive film.
17. A medical dressing for application to the skin of a human subject, the dressing comprising a dressing substrate impregnated or coated with a polymeric molecule according to any one of claims 1-9, or a composition according to any one of claims 10 - 16.
18. A method of treating the skin of a human subject, comprising applying to the skin a composition in accordance with any one of claims 10 - 16.
19. Use of a lipase-degradable polyester according to any one of claims 1 - 9 in the manufacture of a pharmaceutical composition for the treatment of a human skin condition caused or exacerbated by, or associated with, microbial growth.
20. A method of making a cosmetic or pharmaceutical composition for topical application to a human subject, comprising mixing an effective amount of a polymer having a plurality

of repeat units having the structure  $-(O_2C-R-CO_2)-$ , wherein R is a substituted or unsubstituted, unsaturated hydrocarbon radical having 10 or more carbon atoms, with an acceptable carrier substance.

21. A method according to claim 20, wherein the composition comprises a polymeric molecule in accordance with any one of claims 1 - 9.

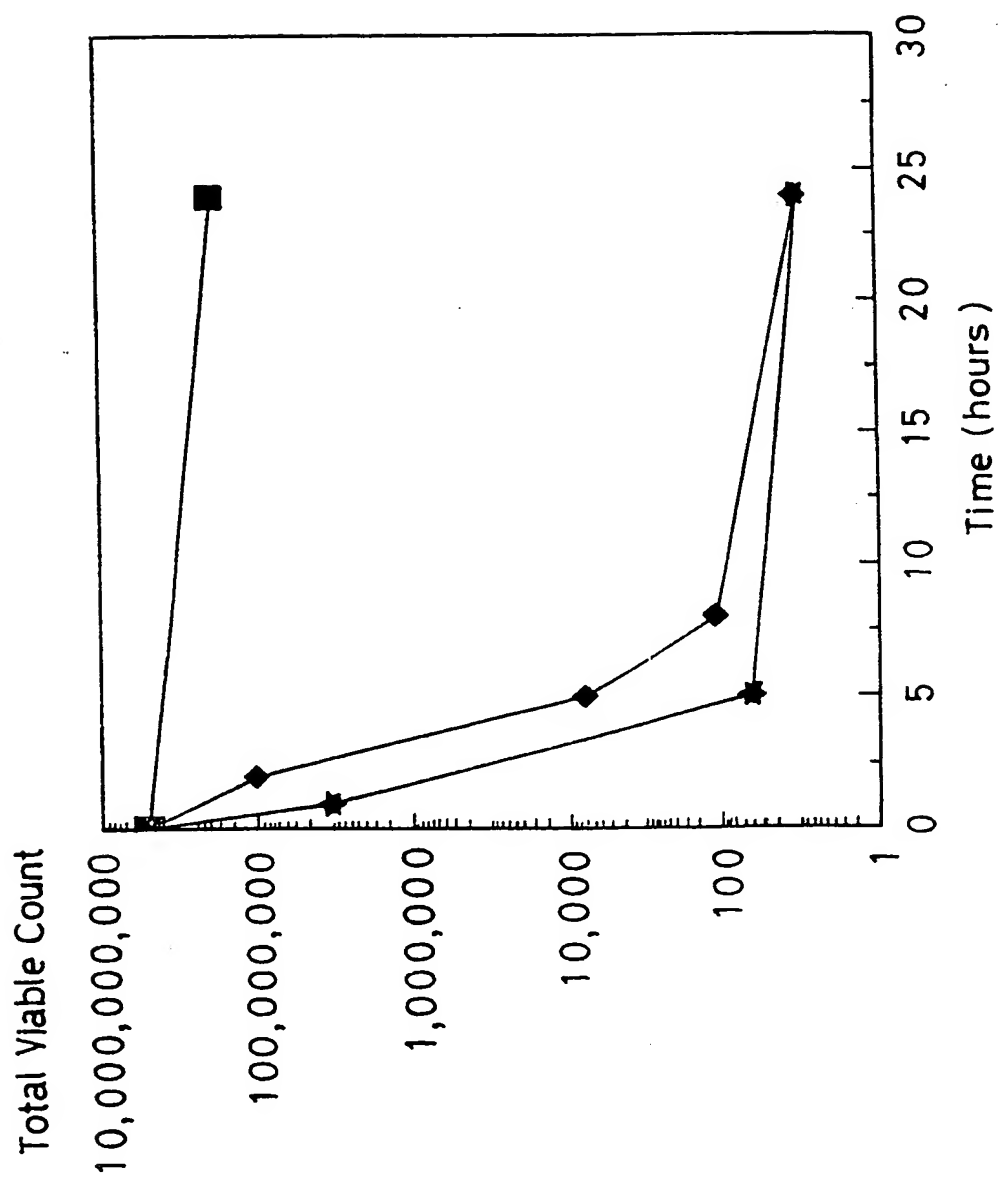
$1/3$ 

Fig. 1

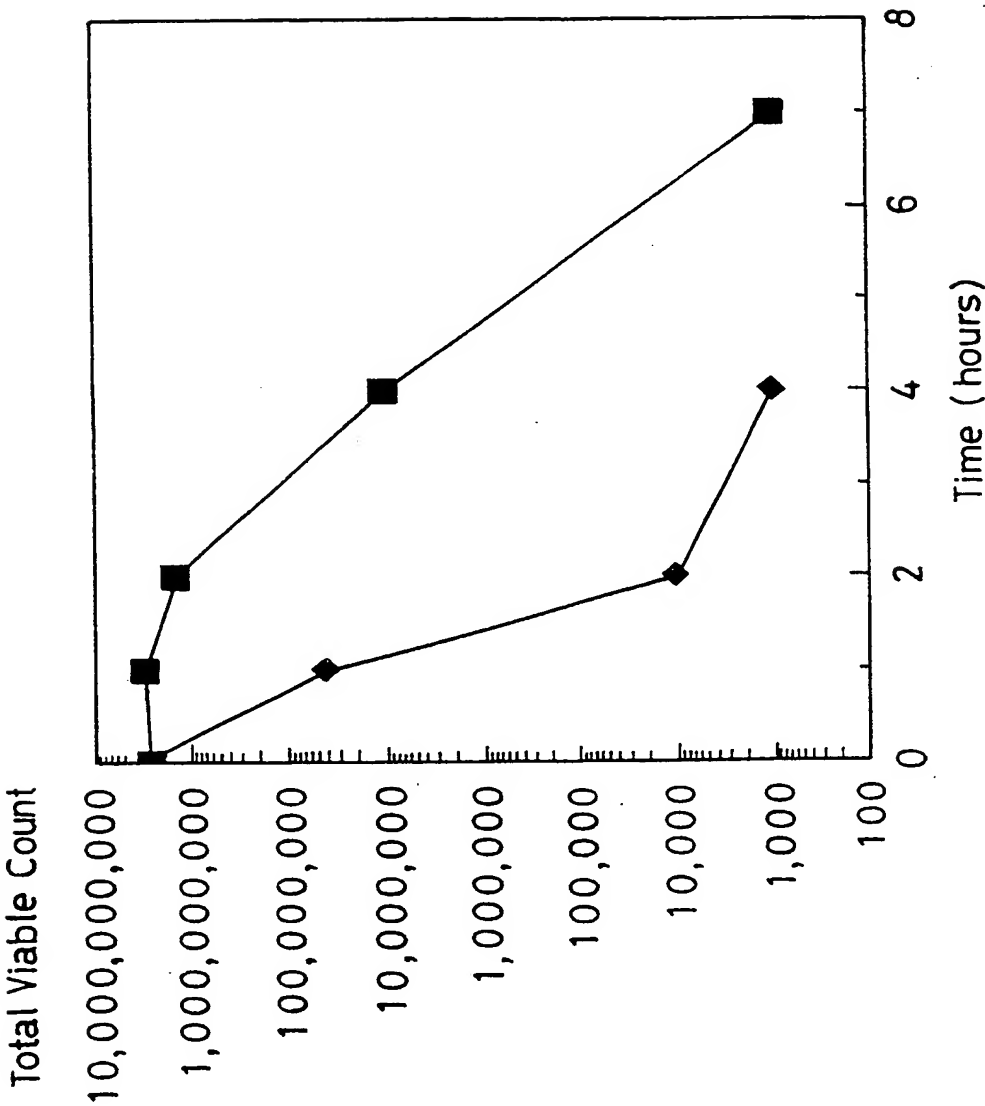
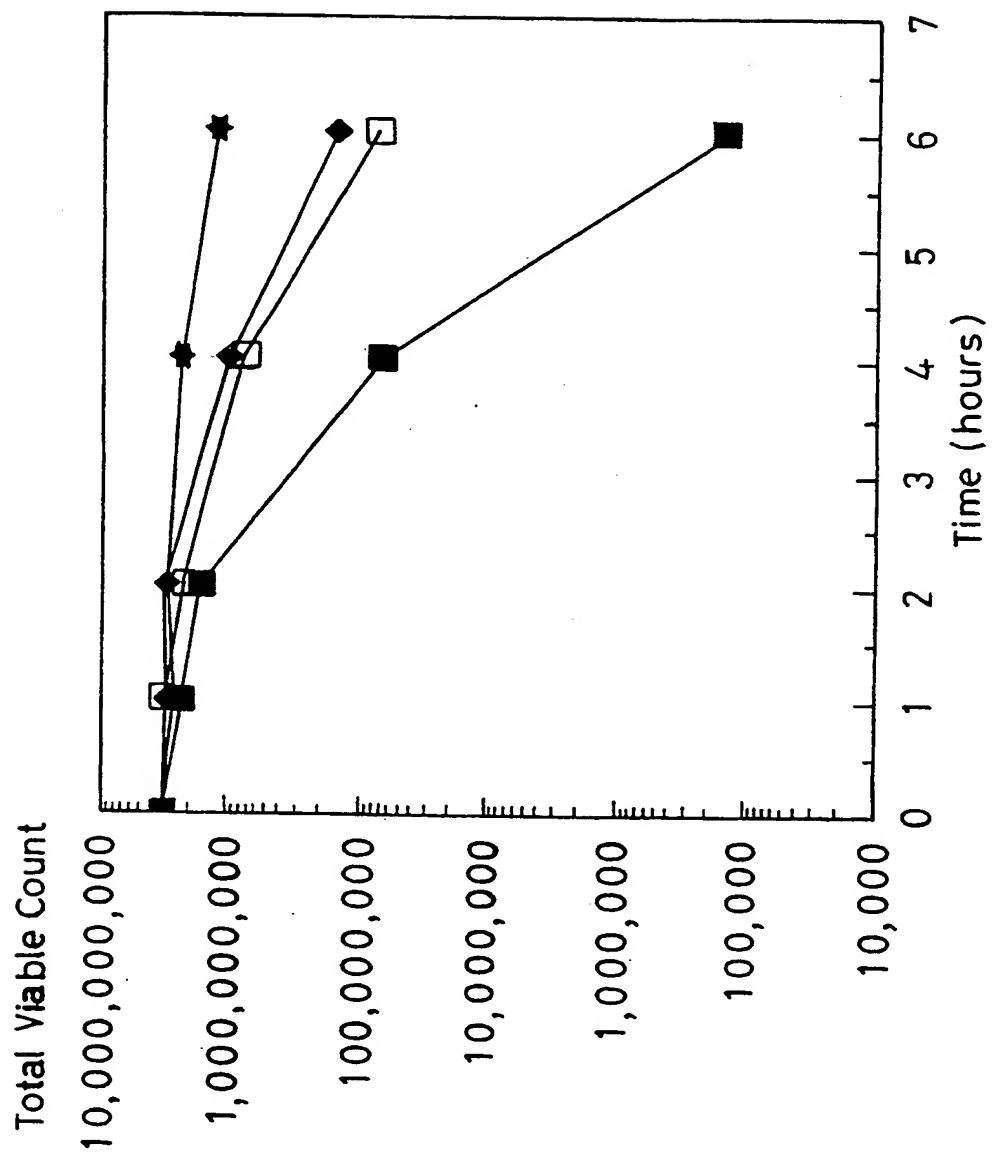


Fig. 2

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/01753

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C08G63/52 A61K7/48 A61K31/215

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C08G A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>Database STN, file CA  Abs. No. 113:85279  &amp;J. Am. Chem. Soc. (1990), 112(15), 5851-4  Jayasuriya, N. et al.  "supramolecular surfactants..."  together with Database STN file reg.  Reg No's 128661-17-6 and 128661-12-1  XP002074857  see abstract</p> <p style="text-align: center;">--- -/--</p>	1-6

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search

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## INTERNATIONAL SEARCH REPORT

Int'l Application No

PCT/GB 98/01753

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database STN, file CA Abs. No. 82:58643 &Farbe Lack (1974), 80(11),1028-9 Van Thiel, J.M. et al. Applications of dicarboxylic..." together with Database STN file Reg Reg. No. 13481-097-5 XP002074858 see abstract ---	1-4,6
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Information on patent family members

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